

**Poster 6. Establishment of a double-haploid production technique using microspore culture for Midwestern U.S. wheat varieties.**

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Double haploids (DH) are genetically 100% homozygous plants. Microspore culture is the method of production of DHs from microspores (immature pollen) by androgenesis in a single step. Success of DH plant production is determined by the genotype and health of the donor plant, environmental conditions under which it is grown, staging of the microspore, pretreatment methods, and composition of induction and regeneration media. The objective of this study is to establish a DH-production technique using microspore culture for the Midwestern U.S. wheat varieties. We used Macon, a Washington spring wheat variety, as a check and three Nebraska winter wheat varieties, Anton, Millennium, and Pronghorn, as representatives of the U.S. Midwest. Plants were grown in the greenhouse with 16 hrs of light at 21–25°C and an 8-hr dark period at 16±2°C. Two pretreatment methods (0.4 M mannitol at 4°C and solution B containing 0.3 M mannitol with inorganic components at room temperature) and one regeneration media was used. We have standardized the staging of microspores and established the steps of the technique (pretreatment, induction, and regeneration) in our laboratory conditions. We were able to produce DH plants for Macon. The microspore culture technique for the winter wheat varieties is in progress. The DH-production technique will be useful in wheat-breeding programs throughout the U.S. Midwest.

**Poster 7. Towards a sequence-ready, physical map of chromosomes 1D, 4D, and 6D of hexaploid wheat.**

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A physical map is a prerequisite for sequence assembly of large genomes such as that of wheat (*Triticum aestivum* L). Because of the high repeat content and genome triplication, assembly of a high-quality, whole-genome sequence may be difficult. Fortunately, flow cytometry allows the division of wheat chromosomes into three fractions based on size (fraction I: 1D, 4D, 6D; fraction II: 1A, 3A, 6A, 2D, 3D, 5D, 7D; and fraction III: 2A, 4A, 5A, 7A, 1B, 2B, 4B, 5B, 6B, 7B) in addition to an individual chromosome 3B. We have developed fraction-I physical maps of chromosomes 1D, 4D, and 6D and three BAC libraries (312,576 clones) from Chinese Spring fraction-I, with a total coverage of 15.3x of the chromosome length. The BAC clones are being fingerprinted with SNaPshot HICF technology. Fingerprinting of first BAC library (*Eco*RI) with 26,112 clones has been completed and fingerprinting of the *Hind*III BAC libraries is in progress. In total, 70,112 clones (3.5x chromosome coverage) have been fingerprinted with a success rate of 96% and are being used for the initial assembly with FPCv9.3. Progress on the assemblies will be discussed. Mapping populations have been developed to anchor, order, and orient the FPC contigs.